Elucidating the mechanisms of action of short-chain fatty acids

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Gut-derived acetate and butyrate increase gluconeogenesis and decrease lipogenesis via the hepatic cAMP-PKA-CREB protein signaling pathway

Gijs den Besten, Aycha Bleeker, Rick Havinga, Albert Gerding, Karen van Eunen, Albert K. Groen, Barbara M. Bakker and Dirk-Jan Reijngoud

Submitted
Abstract

Long-term administration of acetate, propionate and butyrate, the most abundant short-chain fatty acids (SCFAs) of intestinal bacterial fermentation of dietary fibers, has been shown to exert beneficial regulatory effects on glucose and lipid metabolism. The short-term regulatory effects of SCFAs on glucose and lipid metabolism, however, are still unknown. The latter are highly relevant, since SCFA production is not constant but peaks during digestion of a meal. In this study we addressed the regulation during the first 6 hours of exposure of cells and mice to SCFAs. Upon a 6 hour incubation of HepG2 cells with acetate or butyrate, gluconeogenesis increased and lipogenesis decreased. In contrast, propionate only acted as a gluconeogenic substrate. Acetate and butyrate activated the cAMP-protein kinase A-CREB signaling pathway, which then regulated gluconeogenesis and lipogenesis via an increase of peroxisome proliferator-activated receptor γ coactivator 1α and a decrease of peroxisome proliferator-activated receptor γ. This result was independent of AMP-activated protein kinase activation, which is known to be involved in the long-term response. Subsequently, the in vivo effects of 6-hour infusion of SCFAs into the cecum of C57Bl/6J mice were studied. Short-term infusion of acetate of butyrate also activated the hepatic cAMP-PKA-CREB pathway, activated gluconeogenesis and decreased hepatic triglycerides, whereas propionate only increased gluconeogenesis. We conclude that short-term exposure to gut-derived acetate and butyrate increase gluconeogenesis and decrease lipogenesis through activation of the hepatic cAMP-PKA-CREB pathway. In contrast, propionate acts as a substrate for gluconeogenesis only.
Introduction

Dietary fibers are beneficial in the prevention of the metabolic syndrome (1). The main products of bacterial fermentation of dietary fibers in the colon are the short-chain fatty acids (SCFAs), of which acetate, propionate and butyrate are the most abundant. SCFAs exert beneficial effects on host energy metabolism, but our understanding of the molecular mechanisms is far from complete. Long-term administration of SCFAs up to 16 weeks protects against dietary-induced obesity and insulin resistance (17, 122). The protective mechanism for this long-term exposure to SCFAs involves the activation of AMP-activated protein kinase (AMPK) in liver and muscle tissue (17, Chapter 1). Signaling via the G protein-coupled SCFA receptors GPR41 and 43 may be involved because knock-out mice for these receptors develop obesity and insulin resistance over time (135, 215). Short-term SCFA incubation has also been shown to activate AMPK in vitro in hepatocytes and muscle cells (17, 137, 216). However, these in vitro short-term experiments were done in overnight fasted cells. By itself fasting may lead to a low-energy state and a subsequent activation of AMPK and thereby modulate the response to SCFA. The interpretation of short-term effects is further complicated by the rapid metabolism of the SCFAs, which is part of the early response (Chapter 3). In more physiological in vitro experiments, without overnight fasting, addition of SCFAs led to an initial increase of intracellular ATP levels without an effect on AMPK phosphorylation levels (217, 218). Only after 15 hours, the AMP/ATP ratio increased and AMPK was phosphorylated. This indicates that under physiological conditions AMPK activation is a long-term effect and that the short-term regulation mechanisms, if there are any, are still unknown.

In humans, plasma SCFA concentrations vary significantly during the day and peak after meal intake (219, 220). Different types of meals or various dietary fiber concentrations lead to different plasma SCFAs concentrations (221). Together this implies that humans are not exposed to constantly high SCFA concentrations for a long period but rather for short-term bursts. This warrants investigation into the short-term regulatory and metabolic effects.

In this chapter we show that both acetate and butyrate significantly affect gluconeogenesis and lipogenesis through the adenylate cyclase-cAMP-protein kinase A-cAMP response element-binding protein signaling pathway, independently of AMPK activation. These results provide new insights in the physiologically relevant short-term regulatory mechanisms of SCFAs on glucose and lipid metabolism.

Materials and Methods

Animal treatment

Male C57Bl/6j mice (Charles River, L’Arbresle Cedex, France), 2 months of age, were housed in a light- and temperature-controlled facility (lights on 6:30 a.m. to 6:30 p.m., 21 °C) and had free access to water and diet. During 6 weeks mice were fed a semi-synthetic diet (based on D12451 (175), Research Diet Services, Wijk Bij Duurstede, The Netherlands). Experimental procedures were approved by the Ethics Committees for Animal Experiments of the University of Groningen.
HepG2 experiments

HepG2 cells (American Type Culture Collection, Manassas, VA) were maintained at 37°C in 5% CO₂ in DMEM with 10% FBS. For experiments, HepG2 cells were plated in DMEM with 10% FBS in 6-wells plates (657160; Greiner Bio-One) and incubated with 500 µM sodium acetate (S2889; Sigma), sodium propionate (P1880; Sigma), or sodium butyrate (303410; Sigma) with or without 100 µM adenylate cyclase inhibitor dideoxyadenosine (D7408; Sigma) for 6h.

For measurements of glucose production, cells were washed 3 times with PBS and then incubated in glucose free DMEM medium with 10% FBS with 2 mM sodium pyruvate and 20 mM sodium lactate and 500 µM sodium acetate, sodium propionate, or sodium butyrate in the absence or presence of 100 µM dideoxyadenosine. After 6h, glucose levels in the medium were measured with a glucose assay kit (41010; Spinreact).

For measurements of lipogenesis, cells were incubated in DMEM with 10% FBS containing 500 µM sodium acetate, sodium propionate, or sodium butyrate and [³H]H₂O (0.25 mCi/ml) with or without 100 µM dideoxyadenosine. After 6h, fatty acids were extracted and counted for radioactivity as described by Giudetti et al. (222). The incorporation of [³H]H₂O into fatty acids was converted to acetyl equivalents by the factor 1.15 (223).

PKA kinase activity and cAMP concentration

PKA kinase activity (ENZO Life sciences) and cAMP concentrations (R&D systems) were determined in cell lysates and liver homogenates according to the manufacturers' protocols.

Gene expression levels and immunoblot analysis

RNA was extracted from livers using Tri reagent (Sigma-Aldrich, St. Louis, MO) and converted into cDNA by a reverse transcription procedure using M-MLV and random primers according to the manufacturer's protocol (Sigma-Aldrich). For quantitative PCR (qPCR), cDNA was amplified using the appropriate primers and probes. mRNA levels were calculated relative to 36b4 expression and normalized for the control group. The sequence of the primers can be found in the Supplementary Information.

For immunoblot analysis, whole-cell lysates were prepared and mixed with SDS-PAGE loading buffer, heated for 5 min at 96°C and subjected to SDS-PAGE. The immunoblots were analyzed by densitometry using Image Lab software (Bio-Rad). Antibodies used can be found in the Supplementary Information.

Cecal infusion experiment

Mice were equipped with a permanent cecum catheter and allowed a recovery period of at least 5 days as described previously in Chapter 3. Four groups of each 8 mice received either 140 mM sodium chloride (S7653; Sigma), 140 mM sodium acetate (S2889; Sigma), 140 mM sodium propionate (P1880; Sigma) or 140 mM sodium butyrate (303410; Sigma) dissolved in 10 mM phosphate buffer (pH 5.8) infused via the cecum catheter at a rate of 0.2 ml/h for 6h. The infusion rate of SCFAs was based on the recommended intake of dietary fiber for humans of 38 g/day/human, which results in approximately 380 mmol SCFAs/day/human (31, 224). When converted to mice, this corresponds to 170 µmol SCFAs/day/mouse. By infusing 140 mM
SCFAs directly into the cecum at a rate of 0.2 ml/h for 6h, a total amount of 168 µmol SCFA was given per mouse. During cecal infusion, blood glucose concentrations were measured using a EuroFlash meter (Lifescan Benelux) and blood samples were drawn by tail bleeding into heparinized tubes every 2h. After 6h of infusion, animals were terminated by cardiac puncture under isoflurane anesthesia. Cecum content and livers were freeze-clamped and stored at -80 °C.

**Plasma and tissue sampling**

Plasma triglycerides and NEFA concentrations were determined using a commercially available kit (Roche Diagnostics) and plasma insulin levels were determined using ELISA (ALPCO Diagnostics). Hepatic TG content was determined using a commercial available kit (Roche) after lipid extraction (225).

**Statistics**

All data are presented as mean values ± SEM. Statistical analysis was assessed by one-way ANOVA using the Tukey test for post-hoc analysis. Statistical significance was reached at a $p$ value below 0.05.

**Results**

**Acetate and butyrate increase gluconeogenesis and decrease lipogenesis in HepG2 cells**

To investigate the short-term effects of SCFAs on gluconeogenesis and lipogenesis we incubated HepG2 cells for 6h with acetate, propionate or butyrate. In the presence of the gluconogenic substrates lactate and pyruvate, both acetate and butyrate increased glucose production while propionate was without effect (Figure 1A). When propionate was the only gluconogenic substrate, glucose production in the absence of pyruvate and lactate increased from 0.5 ± 0.1 nmol/mg protein in 6h to 1.0 ± 0.1 nmol/mg protein in the presence of propionate, confirming the role of propionate as a gluconeogenic substrate, although not as vividly as pyruvate and lactate (Chapter 3). To test if the SCFA-stimulated glucose production should be ascribed to gluconeogenesis or glycogenolysis, we measured the glycogen content before and after 6h SCFA stimulation in HepG2 cells. Intracellular glycogen was below the detection level, before and after the 6 hour incubation, indicating that the SCFA-induced glucose production was due to gluconeogenesis rather than to glycogenolysis. Lipogenesis decreased upon a short-term incubation with acetate or butyrate while propionate had no effect (Figure 1B). We wondered if the effects on gluconeogenesis and lipogenesis could be attributed to a common mechanism. Phosphorylation of AMPK, which occurs during a long-term incubation with SCFAs, decreases gluconeogenesis and lipogenesis simultaneously (226, 227). This contrasts to the increased gluconeogenesis and decreased lipogenesis that we observed here. In agreement, 6h SCFA incubation had no effect on phospho-AMPK levels (Figure 1C), excluding an involvement of AMPK regulation. We hypothesized that our observations might rather point to activation of the cAMP response element-binding (CREB) protein signaling pathway. Herzig et al. showed in fasting mice that phosphorylation of CREB increased gluconeogenesis by increasing the
expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (228) and decreased lipogenesis by decreasing the expression of peroxisome proliferator-activated receptor γ (PPARγ) (229). When we incubated HepG2 cells with acetate or butyrate, the protein level of PGC-1α increased while that of PPARγ decreased (Figure 1D). In addition, acetate and butyrate increased phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic
Figure 2. Acetate and butyrate regulate gluconeogenesis and lipogenesis via the cAMP-PKA-CREB signaling pathway in HepG2 cells. (A) Active PKA and (B) cAMP concentrations after 6h stimulation with SCFAs. (C) cAMP and (D) active PKA concentrations after 6h stimulation with SCFAs in the presence of the adenylate cyclase inhibitor dideoxyadenosine (ddA). (E) Immunoblot of phospho-CREB and total-CREB after 6h treatment with SCFAs and ddA. Relative phosphorylation levels were calculated by the ratio of phospho-CREB to total-CREB and normalized for control. (F) Immunoblot of PPARγ and PGC-1α expression and their downstream targets after 6h stimulation with SCFAs and ddA. (G) Glucose production in the presence of pyruvate and lactate and (H) lipogenesis upon 6h SCFA stimulation in the presence of ddA. Data represent means ± SEM for n=6; *p<0.05 vs. control.
enzyme and a known target of PGC-1α (230). Incubation with propionate, however, did not affect the protein level of PGC-1α, PPARγ or PEPCK. Propionate induced a minor increase in the protein level of fatty acid synthase (FASN) (Figure 1D). Next we studied the effect of acetate and butyrate on phosphorylation of CREB. Both acetate and butyrate increased the phosphorylation of CREB compared to control, while propionate was without effect (Figure 1E). Apparently, acetate and butyrate, but not propionate, acted via the CREB:PGC-1α/PPARγ signaling pathway.

**Acetate and butyrate increase phospho-CREB through the AC-cAMP-PKA pathway**

Next, we examined how acetate and butyrate increased phospho-CREB levels. CREB is known to be phosphorylated by activation of the protein kinase A (PKA) route (231). Incubation of HepG2 cells with acetate or butyrate increased the activity of PKA (Figure 2A). Because PKA is activated by cAMP, we measured the concentration of cAMP in HepG2 cells, in the absence and presence of SCFAs. Both acetate and butyrate increased the intracellular concentration of cAMP (Figure 2B). Since cAMP is produced from ATP by adenylate cyclase (AC) (232), we evaluated the role of AC in the cascade by studying the effect of the AC inhibitor dideoxyadenosine (ddA) on phospho-CREB and the expression of target genes of CREB. Incubation of cells with ddA abolished the acetate and butyrate-induced increase in cAMP (Figure 2C) and active PKA (Figure 2D). In accordance with our hypothesis that acetate and butyrate activate the AC-cAMP-PKA-CREB signaling pathway, we observed that the increase of phospho-CREB levels during incubation with acetate and butyrate was abolished upon AC inhibition (Figure 2E). Consequently, also the phospho-CREB-dependent increase of PGC-1α and PEPCK, as well as the decrease of PPARγ were not observed anymore (Figure 2F). Finally, also the increase of glucose production and decrease of lipogenesis by acetate and butyrate were blocked by the inhibition of AC (Figure 2G and H). In contrast, the small increase of FASN caused by propionate was unaffected (Figure 2F).

**Infusion of acetate or butyrate into the mouse cecum activates the hepatic cAMP-PKA-CREB protein-signaling pathway in vivo**

To test if the short-term effects of SCFAs on cAMP-PKA-CREB protein signaling that we observed in HepG2 cells were also present in vivo, we infused a physiological amount of SCFAs (see Materials and Methods) directly into the cecum of conscious, unrestrained mice. After a 6h infusion with acetate or butyrate the hepatic cAMP concentrations, active PKA and CREB phosphorylation levels were increased compared to the control or propionate infusion groups (Figures 3A-C). Again, 6h SCFA infusion had no effect on hepatic phospho-AMPK levels (Figure 3D). Next, we examined how the increased phosphorylation levels of CREB affected the expression of its downstream targets PGC-1α and PPARγ. Both acetate and butyrate infusion increased hepatic expression of PGC-1α and its downstream gluconeogenesis target Hnf4α (Figures 3E-F) (233). In addition, acetate or butyrate infusion increased the expression of the PPARγ inhibitor HES1 (229) and decreased that of PPARγ itself (Figures 3E-F).
In vivo activation of the hepatic cAMP-PKA-CREB signaling pathway by acetate and butyrate. (A) Hepatic cAMP and (B) active PKA concentrations after 6h cecal SCFA infusion. (C) Immunoblot of hepatic phospho-CREB and total-CREB after 6h cecal SCFA infusion. Relative phosphorylation levels were calculated by the ratio of phospho-CREB to total-CREB and normalized for control. (D) Immunoblot of hepatic phospho-AMPK and total-AMPK after 6h cecal SCFA infusion. Relative phosphorylation levels were calculated as the ratio of phospho-AMPK to total-AMPK and normalized for control. (E) Hepatic mRNA expression of downstream targets of CREB involved in gluconeogenesis and lipogenesis after 6h cecal SCFA infusion. (F) Immunoblot of hepatic PPARγ and PGC-1α expression after 6h cecal SCFA infusion. Data represent means ± SEM for n=6-8; *p<0.05 vs. control.

In vivo cecal infused SCFAs affect hepatic glucose and lipid metabolism

Next, we wondered how short-term in vivo cecal SCFA infusion and subsequent activation of the cAMP-PKA-CREB signaling pathway affected hepatic glucose and lipid metabolism. After 6h infusion either acetate or butyrate, but not propionate, increased the hepatic mRNA levels of genes involved in gluconeogenesis (Figure 4A). In addition, hepatic PEPCK protein levels were also increased upon acetate and butyrate infusion, similar to what was observed in HepG2 cells (cf. Figures 4B and 1D). With respect to hepatic lipid synthesis, acetate and
butyrate infusion reduced the mRNA expression of Lpl and Cd36, both targets of PPARγ (Figure 4C). Also in agreement with the HepG2 experiments, propionate infusion raised the mRNA and protein levels of genes involved in fatty-acid synthesis (Figures 4C-D and 1D). We further examined if the changes in lipid metabolism had an effect on hepatic triglycerides content. Both acetate and butyrate infusion resulted into a decrease in hepatic triglycerides whereas propionate infusion, despite the increase in genes involved in lipid metabolism, elicited no effect (Figure 4E). There was no effect on hepatic mRNA levels of genes involved in fatty-acid synthesis.
The short-term effects of SCFAs on glucose and lipid metabolism

Figure 5. Cecal SCFA infusion affects metabolite concentrations involved in glucose and lipid metabolism. (A) Plasma glucose concentrations during the cecal SCFA infusion period as percentage of time zero. (B) Hepatic glycogen concentration after 6h cecal SCFA infusion. (C) Plasma insulin concentrations, (D) NEFA concentrations and (E) triglycerides concentrations during cecal SCFA infusion period as percentage of time zero. Data represent means ± SEM for n=7-8; *p<0.05 acetate vs. control, #p<0.05 propionate vs. control, $p<0.05$ butyrate vs. control.

oxidation (Figure 4F), suggesting that the reduced hepatic triglycerides after acetate or butyrate infusion were caused by a decrease in lipogenesis.

**Infusion of SCFAs into the cecum affects whole-body glucose and lipid metabolism**

Finally, we assessed if the altered expression of enzymes involved in hepatic glucose and lipid metabolism resulted into changes in plasma metabolites, reflecting alterations in whole-body physiology. During the 6h infusion period plasma glucose decreased both in control and SCFA infused mice. Cecal infusion of propionate caused the smallest decrease in plasma glucose concentrations during the infusion period (Figure 5A), underlining the importance
of propionate as a gluconeogenic substrate (Chapter 3). In agreement with the increase in expression of gluconeogenic genes in the liver (Figures 4A-B), infusion of acetate or butyrate decreased plasma glucose concentrations less than a control infusion, although not to the same extent as a propionate infusion did (Figure 5A). After 6h SCFA infusion the hepatic glycogen content was increased in all three SCFA-infused groups compared to control mice (Figure 5B). This suggests that SCFAs stimulate glycogen synthesis or at least reduce its consumption. This is a strong indication that the increased plasma glucose levels in the SCFA groups should be ascribed to increased gluconeogenesis rather than to glycogenolysis. We cannot exclude, however, that glucose consumption is also affected. Plasma insulin concentrations decreased during the infusion period with the exception of 2h of propionate infusion for which a significant increase in insulin concentration was observed (Figure 5C). Acetate infusion resulted into a lower plasma insulin concentration than the control whereas butyrate infusion elicited no effect. Neither acetate nor propionate infusion affected plasma non-esterified fatty acids (NEFA) and triglycerides concentrations. In contrast, butyrate infusion raised both plasma NEFA and triglycerides concentrations transiently (Figures 5D-E). Altogether, these results show that the cecal infused SCFAs also affect whole-body physiology and suggest that these changes are, at least, partly caused by the alterations in hepatic glucose and lipid metabolism.

**Discussion**

Long-term administration of SCFAs has been shown to exert beneficial effects on glucose and lipid metabolism, most likely mediated by multi-organ AMPK activation (Chapter 1). However, under physiological conditions humans are exposed to fluctuating SCFA concentrations during the day, which may exert different effects on glucose and lipid metabolism than continuous exposure. The present study showed *in vitro* and *in vivo* that short-term exposure to gut-derived SCFAs activated the hepatic cAMP-PKA-CREB signaling pathway (Figure 6), independent of AMPK.

Incubation of HepG2 cells with acetate or butyrate resulted into significantly higher gluconeogenesis and lower lipogenesis than observed for control or propionate. As AMPK decreases both gluconeogenesis and lipogenesis simultaneously (226, 227), the observed effects cannot be attributed to the activation of the AMPK pathway. This was supported by the fact that there was no change in phosphorylation levels of AMPK upon acetate or butyrate stimulation. Instead, we showed that the changes in gluconeogenesis and lipogenesis were both mediated by the activation of the AC-cAMP-PKA-CREB pathway. The activation of phospho-CREB by butyrate via the AC-PKA pathway has been shown previously in PC12 and Caco-2 cells, model cells used for neurochemical and intestinal epithelial cells, respectively (218, 234, 235). Also, induction of gluconeogenesis via cAMP signaling has been demonstrated in intestinal cells, *in vitro* as well as *in vivo*. However, we are the first to show that both acetate and butyrate directly raise gluconeogenesis and reduce lipogenesis through the AC-cAMP-PKA-CREB pathway in both HepG2 cells and liver tissue.

The mechanism of activation of AC by acetate or butyrate remains unknown. The activity of AC is mainly regulated by ligand binding to G protein-coupled receptors (GPRs). AC is activated via the GPR-coupled G protein $G_{	ext{ins}}$ and inhibited by the G protein $G_{	ext{out}}$ (236). The two
known GPRs that are activated by SCFAs are GPR41 and GPR43, which both signal via the G protein \( G_{\alpha i} \) (Chapter 1). Together with the very low hepatic expression of GPR41 and GPR43, this suggests that acetate and butyrate do not activate AC through GPR41 or GPR43. Whether SCFAs activate AC via other unknown GPRs or through a GPR-independent mechanism remains to be elucidated.

Our \textit{in vivo} results show that type of signaling pathway activated by SCFAs is different, depending on the duration of exposure and the type of SCFA. After 6 hour exposure to acetate or butyrate we observed AC-cAMP-PKA-CREB signaling and concomitant changes of PPAR\( \gamma \) and of PGC1\( \alpha \) in HepG2 cells and mouse liver. This contrasts to the AMPK signaling that has been documented for long-term exposure to SCFAs (17, 137, 216). With respect to the type of SCFA, propionate did not affect the activity of the AC-cAMP-PKA-CREB signaling pathway, but enhanced expression of \textit{Fasn}, \textit{ElovI5}, \textit{Cd36} and protein level of FASN through an unknown signaling mechanism.

The physiological consequences of short-term exposure to acetate and butyrate do not only depend on the measured capacity of gluconeogenesis and lipogenesis. In liver, triglyceride content decreased, which is compatible with the decreased lipogenesis. However, since hepatic triglyceride levels reflect a steady-state between import of fatty acids, synthesis, oxidation and export by lipoproteins (237), we cannot exclude the possibility that decreased import of fatty acids due to the observed reduction of \textit{Lpl} and \textit{Cd36} expression might contribute to the decreased content of triglycerides. With respect to glucose metabolism, CREB signaling in liver was very comparable to that observed in HepG2 cells with increased expression of \textit{Pck1} and \textit{G6pc}, suggesting that also \textit{in vivo} gluconeogenesis is stimulated. In line with this, plasma glucose concentrations decreased less in the SCFA infusion groups than in the control. Moreover, \textit{in vivo} hepatic glycogen content was higher after short-term infusion of acetate, propionate and butyrate compared to control. Possibly, newly synthesized intrahepatic glucose-6-phosphate was partially diverted into glycogen instead of glucose, or glycogen consumption was reduced upon activation of gluconeogenesis (238).

We hypothesize that the acetate- or butyrate-induced activation of gluconeogenesis may contribute to stabilize glucose levels in the postprandial phase. Dietary glucose or glucose...
produced from simple carbohydrates is taken up in the small intestine and maximum plasma glucose levels are reached after approximately 30 minutes after intake (239). As SCFAs are produced by colonic bacterial fermentation from dietary fibers, SCFAs appear later in the bloodstream than the nutrients taken up in the small intestine. Dependent on the fiber type, plasma SCFA levels peak after approximately 2-8 hours after intake (219-221). This means that when plasma glucose levels are not sustained anymore by uptake from the small intestine, SCFAs support activation of and substrate delivery to hepatic gluconeogenesis to maintain euglycemia and at the same time prevent a glucose-overshoot. As a result, intake of dietary fiber leads to extension of euglycemia beyond the decline of glucose absorption from the intestine. The fact that these short-term beneficial effects occur after every fiber-rich meal suggests that it may contribute to the long-term beneficial effects (i.e. decreased body weight, euglycemia and decreased dyslipidemia) of dietary fibers (169).

In conclusion, we show for the first time that the short-term effects of gut-derived acetate and butyrate on glucose and lipid metabolism are mediated through the hepatic cAMP-PKA-CREB pathway, independent from AMPK activation.
Supplemental information

Table 1. Primer sequences used for qPCR.

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Table 2. Antibodies used for immunoblot analysis

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The short-term effects of SCFAs on glucose and lipid metabolism